A STUDY ON THE LARVICIDAL EFFICACY OF FLOWER EXTRACT OF CLITOREA TERNATEA ON SOME MOSQUITO LARVAE AND RELATED EFFECT ON NON – TARGET ORGANISM

SYNOPSIS

Submitted to Ranchi University for the consideration of Ph.D. Registration

IN

ZOOLOGY (Faculty of Science) 2024

SUPERVISOR

Dr. Anjali Smita

ASSISTANT PROFESSOR

DEPARTMENT OF ZOOLOGY,

NIRMALA COLLEGE ,RANCHI

RANCHI UNIVERSITY, RANCHI, JHARKHAND

RESEARCHER

Binita kumari

A study on the larvicidal efficacy of flower extract of *Clitorea ternatea* on some mosquito larvae and related effect on non-target organism

STATE OF THE ART

Insect vectors especially the mosquitoes are obligatory host's of many parasites , and one of the very crucial agents that are capable of transmitting deadly pathogens ,which cause various infectious diseases like dengue, chikungunya, malaria, yellow fever , filariasis etc to humans as well as animals. These diseases posse's the ability to affect the entire community, deteriorating public health and economy of a country. So ,insects are one of the major threats to public health world wide. The World Health organization (WHO) estimates that each year roughly 700,000 – 1,000,000 people succumb to vector borne diseases **(WHO,2024).**

Vector borne diseases transmitted by bloodsucking insects account for over 17% of all infectious diseases, and more than 90% of this fraction is due to mosquito transmitted agents **WHO, Reports (2017) ; Franklinos et al.,(2019); Rocklov and Dubrow et al., (2020).**

These diseases affect more than 200 million people each year and causes more than 400,000 deaths annually , majority of them being children under the age of five. **WHO (2022**). If we consider Dengue which is caused by (*Aedes aegypti)* then ,it is responsible for an estimated 10,000 deaths and 100 million symptomatic infections per year in over 125 countries **Messina et al., (2014) ; Lo'pez et al.,(2018).** Anopheles species are one of the important causative agents of malaria (*Anopheles Stephensi*) which can even be highly severe and fatal disease affecting the liver and red blood cells of humans . A recent WHO report indicates that in 2023, nearly half of the world's population (>3.9 billion) continued to be at risk of the disease, with an estimated morbidity of 249 million and mortality of 608,000 **WHO, World Malaria Report,(2023).**

The expansion of the Mosquito species in different parts of the world is leading to rise in concerns about their control strategies. The rise in global temperature, precipitation patterns and the climate change is causing the insects to adapt a wide range of geographical regions, one of the major challenges of recent times, which could have threatening impacts world wide. In addition to all these, the increase in industrialisation, rapid urbanisation, and the formation of unplanned cities too is posing threat to the increase in the mosquito population. From a microhabitat perspective,

the quality of mosquito larval habitats (breeding sites) is one of the most important determinants of the distribution and abundance of mosquito habitats (**Rejmankova et al.,2013; Laporta et al.,2014; Grech et al.,2019; Chandrasegaran et al.,2020; Nihad et al.,2022).** Several methods have been adopted since many decades to reduce the population of these havoc causing vectors. One among them is the chemical control, Synthetic insecticide are the most used chemicals to kill the larvae and the mosquitoes. There are several insecticides which are employed for the same, such as carbamates , pyrethroids , and organophosphates .The larvae of standing waters are killed by using larvicides . But this approach of controlling the vector is not effective way as it is not easily degradable and remain in the ecosystem for a very longer duration, and gets accumulated in organisms of different trophic levels, affecting the non targeted organisms, not only this but the most of these vectors have developed resistance against these insecticides. The other method involves the physical method, that involves the use of mosquito repellents, mosquito nets, etc and this too is not one of the effective ways of controlling vectors as they are just one of the temporary solutions. After seeing the hazardous effects that are caused by the use of chemical insecticides , researchers are now focusing on plant derived botanicals which can be an alternative for the control of vectors. Nature has gifted us a wide variety of plants, among them there are several medicinally crucial plants, which have amazing properties that are being employed for several purposes. In recent time , scientists around the world are focusing more on the development of safest and sustainable alternatives which will not only kill the target organism , but that should be eco friendly as well , ie. Easily degradable(biodegradable) ,cost effective, and should not affect the non targeted Organisms. Different parts of plants such as leaves, roots, stems, and flowers extract and oils of several plants which possess various important chemicals compounds, that can affect the normal growth, act as a antifeedant substance, and affect the various other activities of the target organism are being assessed. Some of them are *Azadiractin* , *Ocimum sanctum*, *Curcuma longa* , *Gingiber officinalis* etc.

One of the very important plant is *Clitorea ternatea, it* is a medicinally important plant which is used to cure various diseases, and has been used since very long for its well-known properties. *Clitorea ternatea* commonly known as Butterfly pea belonging to the family Fabaceae and sub – family Papilionaceae is a perennial leguminous twinner, which originated from tropical Asia and later was distributed in South and Central America, East and West Indies, China and India, where it has become naturalised **Barik et al.,(2007).**

Mukherjee et al., (2008) investigated *Clitorea ternatea* (Ayurvedic medicine) for its scientific assessment from traditional use. *Clitorea ternatea* is known as Aparajita in Bengali which is used as well- known Ayurvedic medicine. All part of the herb (shoot, leaf, root) is used in medicine. In traditional Ayurvedic medicine, it has been used for centuries as a memory enhancer, anxiolytic, nootropic antidepressant, tranquilizing anticonvulsant, anti - stress and sedative agent.

In line with safer alternative conception, *Clitorea ternatea* plant has the potential as bio-insecticides to solve the problems of resistance from single based chemical compound . *Clitorea ternatea* is a well -known plant in Asian countries such as India , Malaysia, Thailand ,China and widely used in Chinese medicine **Arumugam et al .,(2013)** . This plant is known as Aparajit (Hindi), Aparajita (Bengali), and Kokattam (Tamil) in Indian traditional medicine **Parimaladevi et al.,(2003).** In the traditional (Asian) Indian system of medicine particularly in Ayurveda ,the roots, seeds and leaves of *Clitorea ternatea* have long been widely used as brain tonic and is believed to promote memory and intelligence **Mukherjee et al.,(2007a).**

In recent time ,this plant parts(roots ,stem, leaves, and flowers) are being investigated for its larvicidal activity in different species of mosquitos. Few studies suggest that it is one of the important medicinal plant that can be investigated for its larvicidal activity in different species of mosquitoes. Several investigations have been conducted on leaves ,roots , but only few studies have been conducted so far on flowers of *Clitorea ternatea* ,and those investigation indicates that more studies should be done to examine it's different biochemicals as well as it's effect on the various species of mosquitos. Therefore, the present study aims to evaluate the efficacy of the flower extract of *Clitorea ternatea* on the mosquito larvae of *Aedes* and *Culex.*

National Status

Mosquitoes have developed a lot of resistance against most of the insecticides. The use of botanicals provides a more eco-friendly and cost cutting strategy to combat the mosquito population. Several works have been done to find out more avenues to prepare plant extract to wipe out the mosquito and control their life cycle from very beginning. **Pavananundt et al., (2013)** tested *Cassia siamea* leaf aqueous extract against the *Aedes aegypti* and reported the toxicity and histopathological effects of the *C.* siamea as a bioinsecticide in the midgut. **Pavela and**

Govindarajan., (2017) tested the larvicidal effect of *Zanthoxylum monophyllum* leaf essential oil against non-target, *Gambusia affinis* and found it safer against the non-target fish. **Maurya et al., (2009)** used petroleum ether extract from leaves of *Ocimum basilicum* against *An. stephensi* and *C. quinquefasciatus*. Also, **Senthil Kumar et al., (2009)** reported that, the protein level in *An. stephensi* larvae treated with some phytoextracts was reduced and resumed that it was the result of interference of the plant extracts with normal protein synthesis mechanism. **Chakraborty et al., (2017)** studied phytochemical, biochemical and antimicrobial activities of different plant extract of *Clitorea ternatea*. Phytochemical analysis of the methanolic and aqueous leaf extract of *Clitorea ternatea* showed the presence of flavonoids, carbohydrates, phenols, saponins, tannins, quinines, terpenoids and oxalate components. In aqueous and methanolic extract of seeds presence of alkaloids, carbohydrates, glycosides, flavonoids, tannins, saponins, amino acid, proteins, terpenoids were found . Quantitatively, leaves and seed methanol extracts have good quantity of phenol, carbohydrate, tannin, flavonoids and terpenoids.

Govindarajan et al., (2011) investigated the mosquito larvicidal, ovicidal, and repellent properties of botanical extracts of *Ervatamia coronaria* and *Caesalpinia pulcherrima* against *Anopheles Stephensi, Aedes aegypti,* and *Culex quinquefasciatus (*Diptera: culicidae). The leaf extract of *E. Coronaria* was found to be most effective than *Caesalpinia pulcherrima* against eggs/ egg rafts of three vector mosquitoes. In this observation, these two plant crude extracts gave protection against mosquito bites without any allergic reaction to the test person, and also, the repellent activity is dependent on the strength of the plant extracts.

Ravindran et al., (2020) studied the chemical composition and larvicidal activity of flower extracts of *Clitorea* ternatea against ***Aedes aegypti*** mosquitoes and reported that total of 16 chemical compounds were present in the flower extract which posse's direct effects on larvicidal, insecticidal and pupicidal effects. No toxic effect was observed on non-target organisms.

In the study of alcoholic extract of leaves, flowers and roots of *Clitorea ternatea* gave different pharmacological activities like anti- inflammatory, immunomodulatory, antiasthamatic, anthelminthic, anticonvulsant, antipyretic, antifungal, analgesic, antihyperlipidemic and proteolytic **Gupta et al., (2010)** The scientific research on *Clitorea ternatea* suggests a huge biological activity of this plant. Plants with bioactive compounds have been used to manage different crop pests and human infections with notable success (**Karpagam and Devaraj.,2011 ,**

Thirupalli et al.,2010). Insecticides of botanical origin have been reported as useful for control of mosquitoes. *Azadirachta indica* (Meliaceae) and its derived products have shown a variety of insecticidal properties **Dua et al., (2009).**

International status

Studies have been made globally to resort to such measure that can control the mosquito from early level. Phytoextracts have been highly useful in this regard. Scientist even recommend use of *bacillus thuringenesis* (Bti) as a microbial larvicide for effective result. The protein and carbohydrate determination are a good option to study the underlying mechanism of mosquito larva control. **Valdez et al.,(2021)** examined the insecticidal activity of Butterfly Pea (*Clitorea ternatea***)** leaf extract against mosquito wrigglers and found that both ethanolic and pure leaf extract of *Clitorea ternatea* are effective against mosquito wrigglers.

Semenza et al., (2022) studied the Climate change and cascading risks from infectious diseases and found that climate change is adversely affecting the burden of infectious disease throughout the world, which is a health security threat.

The depletion in carbohydrate content may be due to the utilization of the reserved carbohydrate sources of the larval tissues as a result of plant extract stress **Shehata (2018). Killeen et al.,(2002)** reported a documented example of larval control for sustained and successful malaria prevention in sub-Saharan Africa and this larval control endeavour predates the advent of dichlorodiphenyltrichloroethane (DDT). **Rivero et al., (2006)** investigated the energy budget of *Ae. aegypti* larvae infected by *Vavraia culicis*. For this purpose, they quantified the glycogen, sugars, lipids and protein reserves of infected mosquitoes. **Mathew et al.,(2009)** studied the effect of *Clitorea ternatea* on mosquito larvae and non-target model.

PROBLEM TO BE INVESTIGATED

The spread of mosquito borne illness has been a major concern for humanity since long span of time. Chemicals and pesticides have been developed to control their growing population. But it

has always been a costly method with severe repercussion on environmental well- being. Hence plants can provide better and valid results if they are used as a deterrent to growth of mosquito larvae. The present study will try to study efficacy of flower extract of *Clitorea ternatea* on mosquito larvae as a potential larvicide. Moreover, the efficacy of the flower extract will also depend on its effect on the non- target organism. It will only be effective if its non- damaging to the non- target organisms.

OBJECTIVE OF THE INVESTIGATION AND MOTIVATION

Mosquitoes have developed a lot of resistance to the conventional insecticide. The repeated use of insecticides depletes the natural reserves of soil and water. Hence flower extracts of *Clitorea terneata* can be an option of combating the mosquito in its larval stage.

a) Preparation of aqueous and methanolic extract of flowers of C*litorea ternatea* will be done
b) Investigation of chemical composition of flower
c) LC 50 will be determined
d) Water quality analysis of test water
e) Study of bioassay and enzyme activity in larvae
f) Investigation to ascertain effect of the flower extract on non-model organism.

HYPOTHESIS

Synthetic insecticides have been the major tool in mosquito control operations. However, the extensive and repeated use of synthetic organic insecticides such as organophosphates and organochlorines have led to disrupted natural biological control systems heading to resurgence and resistance in target species and destruction of nontarget beneficial fauna, resulting in fostered environment and human health concern. One of the safest methods of control of insect pests and vectors is through the application of easily degradable plant compounds. Flower Extract of *Clitorea ternatea* could be a good alternative for control of mosquito in their juvenile form. It will be easy to handle and control the larval forms of mosquito as compared to its adult forms. It will help in making use of the plant for the benefit of the society. Moreover, it will give an impetus to plant more plants of *Clitorea ternatea* which has wide medicinal properties.

RESEARCH DESIGN AND METHODOLOGY

I Experimental Animal

Culex and *Aedes* mosquito larvae will be taken for the experiment;

II Study area

The study will be carried out in Ranchi (23.35° N latitude, 85.33°E longitude) located in southern part of Chotta Nagpur region.

III Larval sampling:

Mosquito larvae will be collected from various localities of Ranchi and all instars will be maintained in the laboratory following the method used by WHO (**Ravi et al.,2018; Zulkrin et al., 2018; Ravi et al., 2018; Ravi et al., 2020 and WHO 2005)** in larvae rearing. The eggs will be kept in dechlorinated water for 24 hours maintained at 25° C to 30° C (room temperature), pH of 6.95 to 7.03, and relative humidity of 80 ± 10% and dissolved oxygen from 5.5 to 6.1 mg/L in the laboratory. After five days, the early 4th instar larvae will be used for the bioassay test. Level of pH and dissolved oxygen will be checked in test water samples.

IV Preparation of plant extract

Aqueous and Methanolic extract of *Clitorea ternatea* will be prepared to study their effect on the growth and longevity of the mosquito larvae.

The petals of *C. ternatea* will be washed in running tap water to remove adhered debris and soil particles and washed again with distilled water and dried in hot air oven at 70°C until the petals are completely dry **Wong and Williamkoh., (2006).** The dried petals were ground into fine powder by using a laboratory grinder and stored in a vacuum-tight container for further usage.

V Types of Extraction Preparation

Aqueous Extraction. Aqueous extraction was performed by maceration using 250 ml distilled water and 50 g of the powdered *C. ternatea* flowers petal. They were mixed in a 500 ml beaker

and stored in a dark area for an hour. Then, the extract was filtered using cotton wool and filter paper. The final volumes were stored in a universal bottle and maintained at 4°C to 6°C **Wong and Williamkoh.,(2006).**

Methanol Extraction. Methanol extraction will be performed in three different concentrations of 50%, 70%, and 95%. A mixture of 5 g powdered *C. ternatea* flower petals and 100 ml of respective concentration of methanol was prepared in three separate Scott bottles and covered with aluminium foil to avoid exposure to light. Later, the bottles were kept overnight in the orbital shaker for the maceration process. After 24 hours, the extract was filtered using cotton wool and filter paper by vacuum filtration method. Subsequently, they shall be concentrated using rotary evaporator at the temperature of 45°C. Finally, the remained concentrated amount of extract was stored in a universal bottle and maintained at 4°C to 6°C (**Ravi et al.,2018; Anand et al.,2011).**

VI Phytochemical analysis of the plant extract

Crude extracts of the petals will be subjected to phytochemical analysis **Harborne (1984); Trease et al.,(1989); Sofowora et al.,(1993) .**

VII Larvicidal bioassay

Larvicidal bioassays will be carried out according to the guidelines given by World Health Organization **WHO (2005).** The larvicidal tests will be conducted in 250 ml of deionized water. For each test concentration 20 fourth instar larvae will be used. For experimental treatment the stock solutions of the flower extract larvicides will be prepared. LC_{50} will be calculated at different concentration. After giving 24 and 48 hours of sublethal exposure fourth instar larvae will be collected from experimental and control concentrations to determine the glycogen content and total protein estimation to determine the possible mode of action leading to the larval mortality. Acetylcholinesterase activity will also be checked during the study.

a) Analysis of total carbohydrate content

The method of analysis will follow procedure as described by **Singh and Sinha (1977).** Body homogenate of ten mosquito larvae will be used for the bioassay,

b) Analysis of total protein content

The total protein content will be determined using folin phenol reagent according to the method of **Lowry et al., (1951).** Quantitative alterations in protein samples would be done by SDS-PAGE.

c) Determination of Acetyl cholinesterase activity

The activity of acetylcholinesterase (AChE) was determined by the method of **Ellman et al., (1961).**

VIII. Effect on non-target organism

Guppy fish, *Poecilia reticulata* will be used to study the effect of *C ternatea*. A total of five fish in three replicates will be taken for study to assess the toxic effect of the flower extract.

IX Statistical analysis: Results of biochemical studies will be analysed statistically. From every experimental data mean and std deviation will be calculated. To test the significance t test or ANOVA will be used for both treated and control group.

YEAR WISE PLAN OF WORK AND TARGET TO BE ACHIEVED

Stage I

a) Collection and acclimation of Mosquito larvae
b) Extract preparation of flower of Clitorea ternatea
c) Phytochemical analysis of flower extract
d) Exposure of flower extracts to mosquito larvae

Stage II

a) Bioassay of mosquito larvae
b) Effect on non-target organisms

c) Statistical calculation

Stage III

a) Thesis writing

TOOLS TO BE USED IN COLLECTION OF DATA

Aquatic net

Plastic trays

Water analysis tools

Glassware

Spectrophotometer/Colorimeter

Soxhlet

SIGNIFICANCE OF THE STUDY

The flower extract of *Clitorea ternatea* is used in Ayurveda due to its healing properties. Therefore, based on earlier work and research it can be used to study larvicidal against mosquito larvae. It would help to understand the mechanism of action on the larvae that can be effective in controlling them at the initial larval stages rather than to control them in the adult stage which is mainly based on chemical control.

RELEVANCE TO THE PRESENT-DAY PROBLEM AND NEED OF THE SOCIETY

The use of pesticides and synthetic insecticides has not only depleted the natural resource but have also led to the development of resistance among the mosquito population. Non-toxic, biodegradable and easily available plant material can be a better alternative to control the unlimited growth of mosquito juveniles that after transforming to adults become notorious for biting and spreading vector borne illness. It will be also pivotal for the common mass to use such pro-environmental methods to keep away mosquito.

CONTRIBUTION TO THE EXISTING KNOWLEDGE

Chemical control of mosquitoes has led to several hazards to the environment and ecosystem. In order to combat these devastating effects of insecticides and pesticides we need to explore the natural products which Chemical insecticides for mosquito control have become a daily lifestyle that makes the control of mosquitoes more complicated due to the increased resistance to these synthetic compounds. Therefore, new materials from the natural origin are needed to avoid the hazards of these synthetic chemicals. One of the safest methods of control of insect pests and vectors is through the application of easily degradable plant compounds **Rajput et al., (2020).** plant based insecticides will be suitable and adaptive to local ecological conditions, biodegradable and in the future may act as a suitable alternative product to fight against mosquito-borne diseases.

FUTURE SCOPE

Extracts from plants may be alternative sources of mosquito control agents because they constitute a rich source of bioactive compounds that are biodegradable into nontoxic products and potentially suitable for use to control mosquitoes. Insecticides of botanical origin may serve as suitable alternative biocontrol techniques in the future. In view of the recently increased interest in developing plant origin insecticides as an alternative to chemical insecticide, the study can be helpful in finding better solution to control mosquito larvae. In case of beneficial results local people and folks can use it to control mosquito wrigglers which will be both cost effective and eco - friendly. Moreover, commercialising the plant products could also generate employment among masses.

BIBLIOGRAPHY

Anand, S., Doss, A. and Nandagopalan, V.(2011) "Antibacterial studies on leaves of Clitoria ternatea linn.—a high potential medicinal plant," International Journal of Applied Biology and Pharmaceutical Technology, vol. 2, no. 3: pp. 453–456, 2011.

Arumugam, G., Manjula , P., Paari , N. (2013). A review: Anti diabetic medicinal plants used for diabetes mellitus. J Acute Dis 2: 196-200.

Barik, D. P., Naik, S. K., Mudgal, A., Chand, P. K. (2007) Rapid plant regeneration through in vitro axillary shoot proliferation of butterfly pea (*Clitorea ternatea* L.) a twinning legume, In vitro Cell. Dev. Biol. – Plant, 43: 144 -148.

Chakraborty, S., Sahoo, S., Bhagat, A., Dixit, S. (2017). Studies on Antimicrobial activity, phytochemical screening tests, Biochemical evaluation of *Clitorea ternatea* Linn. Plant extracts. International Journal Of Research Granthaalayah vol.5: (Iss.10) ISSN- 2350-0530(O), ISSN-2394-3629 (P), DOI: 10.5281/zenodo.1040675.

Chandrasegaran, K., Lahondere, C., Escobar, L.E., & Vinauger, C. (2020). Linking mosquito ecology, traits, behaviour, and disease transmission. Trends in parasitology. 2020; 36(4): 393-403. https://doi.org/10.1016/j.pt.2020.02.001 PMID: 32191853.

Dua, V.k., Pandey, A.C., Raghavendra, k., Gupta, A., Sharma, T., & Dash, A.P. (2009). Larvicidal activity of neem oil (Azadirachta indica) formulation against mosquitoes. Malaria Journal 2009, 8:124 doi: 10.1186/1475-2875-8- 124.

Ellman, G. L., Courtney, K. D., Andres, V., JR . & Featherstone, R.M. (1961) A new and Rapid Colorimetric Determination of acetyl cholinesterase activity, Biochemical Pharmacology, vol 7: 88 – 95.

Franklinos, L.H.V., Jones, K.E., Redding, D.W. and Abubakar , I. (2019). The effect of global change on mosquito- borne diseases. Lancet Infect Dis 19, 9-e312 .

Grech, M.G., Manzo, L.M., Epele, L.B., Laurito, M., Claverie , A.N., Luduena- Almeida, F.F,…& Almiron, W.R. Mosquito (Diptera:Culicidae) larval ecology in natural habitats in the cold temperate Patagonia region of Argentina. Parasites & Vector . 2019; 12(1): 1-14. https://doi.org/10.1186/s13071-019-3459-y PMID: 31064397.

Govindarajan, M., Mathivanan, T., Elumalai, K., Krishnappa, K., Anandan, A. (2011). Mosquito larvicidal, ovicidal, and repellent properties of botanical extracts against *Anopheles stephensi, Aedes aegypti,* and *Culex quinquefasciatus* (Diptera: Culicidae). Parasitol Res. 109: 353 – 367.

Gupta, G. K., Chahal, J., Bhatia, M. (2010). *Clitorea ternatea* (L.) : Old and new aspects , Journal of Pharmacy Research, 3(11): 2610 – 2614.

Harborne, J.B. Phytochemical methods. A guide to modern techniques of plant analysis. London: Chapman and Hall; 1984: p. 49-188.

Killeen, G.F., Fillinger, U., Knols, B. G., Advantages of larval control for African malaria vectors: low mobility and behavioural responsiveness of immature mosquito stages allow high effective coverage. Malaria Journal. 2002;1: 8. doi: 10.1186/1475-2875-1-8.

Karpagam, T., Devaraj, A.(2011). Studies on the efficacy of aloevera on antimicrobial activity, Int. J Res Ayurveda anad pharma: 1286 - 89

Lo'pez, M. S., Muller, G. V., Sione, W. F. Analysis of the spatial distribution of scientific publications regarding vector – borne diseases related to climate variability in South America. Spot spatiotemporal Epidemiol. 2018, 1(26): 35-93.

Laporta, G.Z., Sallum, M.A. (2014). Coexistence mechanism at multiple scales in mosquito assemblages. BMC Ecology. 2014; 14, 30. https://doi.org/10.1186/s12898-014-0030-8 PMID: 25384802.

Lowry, O.H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein Measurement with a Folin Phenol reagent. J Biol Chem, 193(1): 265 – 75.

Maurya, P., Sharma, p., Mohan, L., Batabyal, L., Srivastava, C.N. (2009). Evaluation of the toxicity of different phytoextracts of Ocimum basilicum against Anopheles stephensi and Culex quinquefasciatus. Journal of Asia-Pacific Entomology, 12: 113- 115.

Messina, J. P., Brady, O. J., Pigott, D. M., Brownstein, J. S., Hoen, A. G., Hay, S. I. A global compendium of human dengue virus occurrence. Sci Data. 2014, 1(1): 1-6.

Mukherjee, P. K., Kumar, V., Kumar, N. S., Heinrich, M. (2008). The Ayurvedic medicine *Clitorea ternatea* – From traditional use to scientific assessment. Journal of Ethanopharmacology, 120: 291-301.

Mathew, N., Anitha, M.G., Bala, T. S. L., Shivakumar, S. M., Narmadha, R., Kalyanasundaram, M.(2009). Larvicidal activity of *Saraca indica, Nyctanthes arbor – tritis, and Clitorea ternatea* against three mosquito vector species. Parasitol Res 104: 1017 – 1025.

Mukherjee, P.K., Kumar, V., Mal, M., Houghton, P.J., 2007a. Acetyl cholinesterase inhibitors from plants. Phytomedicine 14: 289-300.

Nihad, M., Rohini, P.D., Sutharsan, G., Anagha, A.P.K., Sumitha M.K., Shanmuga Priya, A., Rahul, P., Sasikumar, V., Dasgupta, S., Krishnan, J. and Istiaq, F. (2022). Island biogeography and human practices drive ecological connectivity in mosquito species richness in Lakshadweep Archipelago. Scientific Reports. 2022; 12: 8060.

Pavananundtt, P., Jiraungkoorskul, K., Kosai, P. and Jiraungkoorskul, W. (2013). Larvicidal properties of Cassia siamea leaf against Aedes aegypti larvae. Int. J. Modern Agric, 2(1): 1-8.

Parimaladevi, B., Boominathan, R., Mandal, S. C.(2003). Anti – inflammatory, analgesic and antipyretic properties of *Clitorea* ternatea root, Fitoterapia, 74: 345 – 349.

Pavela, R. and Govindarajan, M. (2017). The essential oil from Zanthoxylum monophyllum a potential mosquito larvicide with low toxicity to the non-target fish Gambusia affinis. Journal of Pest Science, 90(1): 369-378.

Rajput, VS., Jhala, J., Acharya, V. (2020). Biopesticides and their mode of action against insect pests: A review. International journal of chemical studies, 8(2): 2856-62.

Ravi, R., Zulkrnin, H., Shaida, N. (2018). "Evaluation of two different solvents for Azolla pinnata extracts on chemical compositions and larvicidal activity against Aedes albopictus (Diptera: Culicidae)," Journal of Chemistry, vol. 2018, Article ID 7453816: 8 pages.

Ravi, R., Zulkrnin, N. S. H., Rozhan, N. N. (2018). "Chemical composition and larvicidal activities of Azolla pinnata extracts against Aedes (Diptera: Culicidae)," PLoS One, vol. 13, no. 11, Article ID e0206982.

Ravi, R., Ishak, I. H., Mohd Amin, M. F., Rasat, M. S. M., Salam, M. A., Mohd Amin, M. A.(2020). "Nano-synthesized silver particles and Azolla Pinnata extract Larvicidal effects against aedes aegypti (diptera: culicidae)," International Journal of Advanced Science and Technology, vol. 29, no. 4: pp. 3420–3427.

Rejmankova, E., Grieco, J., Achee, N., Roberts, D.R. Ecology of larval habitats. In: Manguin S, editor. Anopheles mosquito: new insights into malaria vectors 9th.2013 In Tech; Rijeka: pp. 397-446.

Rivero, A., Agnew, P., Bedhomme, S., Sidobre, C. and Michalakis, Y. (2007). Resource depletion in Aedes aegypti mosquitoes infected by the microsporidia Vavraia culicis. Parasitology. 134 : 1355-1362.

Ravindran, D. R., Bharathithasan, M., Ramaiah, P., Rasat, M. S. M., Rajendra, D., Srikumar, S., Ishak, I. H., Said, A. R., Ravi, R., Amin, M. F. M. (2020) Chemical composition and larvicidal activity against *Aedes* mosquitoes of flower extract from *Clitorea ternatea.* bioRxiv preprint : https://doi.org/10.1101/2020. 03. 08465.

Rocklov, J. and Dubrow, R. (2020). Climate change: an enduring challenge for vector- borne disease prevention and control. Nat Immunol 21: 479-483.

Senthilkumar, N., Varma, P., Gurusubramaniam, G., (2009). Larvicidal and adulticidal activities of some medicinal plants against the malaria vector, Anopheles stephensi (Liston). Parasitology Research, (104): 237-244.

Semenza, J. C., Rocklov, J., Ebi, K. L. (2022) Climate change and cascading risks from infectious disease. Infect Dis Ther 11: 1371 – 1390.

Shehata, A.Z.I., 2018: Repellent Activity of Deverra triradiata (Apiaceae) extracts against Anopheles sergentii Theobald, Culex pipiens Liston and Culex antennatus Becker mosquitoes. Journal of the Egyptian Society of Parasitology 48(3): 599-604.

Singh, N.B., Sinha, R. N. (1977). Carbohydrates, lipids and proteins in the developmental stages of Sitophilus oryzae and S. granaries (Coleoptera: Curculionidae). Annals of the Entomological Society of America,70: 107-111.

Sofowora, L.A. Medicinal plants and traditional medicine in Africa. Ibadan, Nigeria: Spectrum Books; 1993: p. 55- 71.

Thirupalli, S., Ramasubramanian, V., Sivakumar, T., Thirumalai , A. V.(2010). Antimicrobial activity of Aloe vera (L)Burm.f. against pathogenic microorganisms..J.Bios Res.4(2010): 251-258.

Trease, G.E., Evans, W.C. Pharmacognosy: a physician's guide to herbal medicine, 13th ed. London: Bailliere Tindal, (1989).

Valdez, A., Abusama, H., Falle, J., Lapuj, N., Martinez, S., Pineda, S., Tolentino, A. (2021) Insecticidal effects of Butterfly pea (*Clitorea ternatea)* leaf extract against mosquito wrigglers. ASEAN journal of Science and Engineering 1 (3): 141 -144.

Vector Born Diseases- World health organization (WHO). Available online: https://www.who.int/news-room / fact-sheets/details/ vector borne diseases (accessed on 15 Feb 2024).

WHO, Global Vector Control Response 2017-2030. World Health Organization (WHO), Geneva (2017).

WHO. 2022. World malaria report 2022. Geneva: World Health Organization , License: CC BY – NC – SA 3.0 IGO: 293.

WHO 2023 . World Malaria Report 2023 . Available online: https:// www.who.int/ teams / global-malaria-programme / reports / world – malaria- report -2023 (accessed on 15 February 2024).

World Health Organization, Guidelines for Laboratory and Field Testing of Mosquito Larvicides, World Health Organization, Geneva, Switzerland, 2005.

Wong, S., Leong, L. and Williamkoh, J.(2006). "Antioxidant activities of aqueous extracts of selected plants," Food Chemistry, vol. 99, no. 4: pp. 775–783.

Zulkrnin, H., Shaida, N., Rozhan, N. N. (2018). "Larvicidal effectiveness of Azolla pinnata against Aedes aegypti (Diptera: Culicidae) with its effects on larval morphology and visualization of behavioural response," Journal of Parasitology Research, vol. 2018, Article ID 1383186: 5 pages.